Uropathogens And Antimicrobial Susceptibility Pattern In Adult Patients Attending A Tertiary Hospital In Enugu, Nigeria

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Abstract
Background: Urinary tract infections (UTIS) are prevalent in clinical practice. Treatment options are narrowing down because of multidrug resistance (MDR) nature of some uropathogens.

Objectives: To identify the bacterial etiology of urinary tract infections (UTI) among adult patients attending the University of Nigeria Teaching Hospital (UNTH) Ituku Ozalla Enugu and their antibiotic susceptibility pattern with emphasis on multidrug resistance (MDR).

Materials and methods: the study was carried out on 260 adult urine samples in bacteriology laboratory of UNTH from March to November 2017. Standard microbiological methods were used to identify the isolates and the antimicrobial sensitivity testing was done using Kirby Bauer disc diffusion method.

Results: Of the 260 urine samples analyzed, 96 yielded significant bacteria giving an overall prevalence of 36.92%. Gram negative bacteria were predominant 81(84.4%). Escherichia coli (32.29%) ranked highest and the least was Acinetobacter spp (1.04%). Antimicrobial susceptibility testing showed that majority of the isolates of E. coli were susceptibility to Nitrofurantoin, Meronem, Tigecyclin, Imipenem and Ciprofloxacin (71-93.5%). Most of the isolates showed high level of resistance to Augmentin, Cefuroxime, Cefazidine and Carbanicillin. Prevalence of MDR was high in E.coli, 12(27.3%) isolates produced ESBL while 4(28.6%) isolates of Staphylococcus aureus were methicillin resistant

Conclusion: Gram negative bacteria are the major cause of UTI and antimicrobial resistance had become a significant public health problem in Nigeria. Regular surveillance of antimicrobial susceptibility profile is imperative.

INTRODUCTION
Urinary tract infections (UTIs) are prevalent in clinical practice [1]. It is the second most common infection after respiratory tract infection [2] and the most important cause of morbidity in the world, affecting all age groups and both gender. It usually requires urgent medical attention [3]. Nearly 150 million people are diagnosed with UTI every year costing global economy in excess of 6 to 8 billion dollars [4, 5].

Urinary tract infection is the presence of pathogens in any part of the urinary tract. UTIs are mostly classified based on the location of the infection as bladder (cystitis), kidney (pyelonephritis) or urine (bacteriuria). They may be asymptomatic or symptomatic [6]. The symptoms of UTI are vague and may lead to muzzy diagnosis [7].

UTI constitutes a serious health risk because of the fact that it may lead to urosepsis and or renal scarring, progressive kidney damage with associated high mortality morbidity and economic loss [8, 9].

Numerous microorganisms may cause UTIs but the most prevalent pathogens causing the simple ones in the community setting are Escherichia coli and other
Enterobacteriaceae which account for almost 75% of the isolates [6, 10]. Women are more susceptible than men because of short straight anatomy of the urethra and termination of female urethra beneath the labia resulting in colonization of colonic gram negative bacilli [7].

Therapy of UTI is often started empirically and treatment is based on information obtained from the antimicrobial resistance pattern of the urinary pathogen [6]. Resistance of pathogenic organisms to overcome antimicrobials is now a global problem with grave consequences on the treatment of infectious diseases. The abuse of antimicrobials in medicine and agriculture is contributing to this resistance. There is a startling increase in antibiotic resistance of bacteria that cause either nosocomial infections or community acquired infections. Worthy of note are the multidrug resistant pathogens like Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii and meticillin-resistant Staphylococcus aureus [11, 12].

The changing trend of antibiotics resistance has made it pertinent to research into susceptibility profile of commonly used antibiotic at various intervals to guide in clinical management of the infection [13]. Hence, the purpose of this study was to determine the etiological agents of UTI and their antimicrobial susceptibility profile among adults attending the University of Nigeria Teaching Hospital (UNTH) Enugu Nigeria with emphasis on MDR (multidrug resistance).

MATERIALS AND METHODS

Study Design

The study was carried out in bacteriology laboratory of UNTH from March to November 2017. It was a cross-sectional study hospital based study. UNTH is a referral hospital and one of the largest teaching hospitals in Nigeria.

Sample collection

Freshly voided midstream urine samples were collected aseptically from 260 in and out patients from 18 years and above with signs and symptoms suggestive of UTI. The samples were received in wide mouthed sterile universal containers labeled with serial number, age, and sex of the patients.

Laboratory diagnostic methods

Cystine lysine electrolyte deficient (CLED) agar and blood agar plates (Oxoid) were used for the inoculation of the urine samples using sterile, calibrated standard wire loop (0.002ml). The plates were incubated at 370c for 24-48 hours and observed for growth. At the end of the incubation period, colony counts were determined. Urine samples having over 105 CFU per milliliter were recorded as significant [14]. The isolates were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method using CLSI criteria on Mueller-Hinton agar (MHA) [15]. The defining criterion for MDR in our study was resistance to more than two antimicrobial agents of different chemical structure [16]. The isolates were identified using colonial morphology, Gram stain and standard biochemical tests.

The E.coli isolates were screened for ESBL according to CLSI standard using ceftazidine (30g) and cefotaximie (30g) [17]. ESBL confirmation tests were done on the positive isolates using different combination discs with ceftazidine clavulanate and cefotaxime clavulanate spectrum B-lactamase (ESBL) detection discs.

Interpretation

Isolates showing ceftazidine <22mm and cefotaxime <27mm were the potential ESBL producing strains. All strains of Staphylococcus aureus that were resistant to cefoxitin invariably become resistant to all β-lactam antibiotics including penicillins, cephalosporins, and cabapenems [18].

Data analysis

Statistical package for social science (SPSS), software version 20 and Microsoft Excel 2007 were used to analyze data and P < 0.05 was considered to be statistically significant.

Ethical approval

Ethical committee of UNTH, Ituku-Ozalla, Enugu, Nigeria approved the study and informed consent was obtained from the patients.

RESULTS

Urine samples were collected from 260 adult patients from 18 years and above. Of the 260 urine samples, 96(35.8%) showed significant bacteria (>105 CFU/ml) whereas 164 (63.1%) were negative. From the 96 positive cases, 96 single pathogens were recovered, 15.63% were Gram positive while 84.38% were Gram negative. The most dominant
microorganisms amid the Gram-negative pathogens were E.coli 44(45.83%), followed by
Proteus 11(11.46%); and the least was Acinetobacter spp. 1 (1.04%). S. aureus was the only Gram positive organism isolated 15 (15.63%) (Table 1).

Most of the isolates showed increased rates of resistance to augmentin, cefuroxime, ceftazidine and carbanicillin.

S. aureus showed high susceptibility to tigecyclin and ceftriaxone. Gram-negative bacteria also exhibited high susceptibility to nitrofurantoin, meronem and impenem as shown in Table 2.

The prevalence of MDR in E.coli was 27.3% while 28.6 % were meticillin resistant (Table 3). Of the 260 adult patients sampled and examined, 96 single organisms were isolated giving a prevalence rate of 36.92%.

There was a higher prevalence in female 24.23% as against 12.69% in males and this was find to be statistically significant P<0.05 (Table 4).

### Table 1

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>44(45.83%)</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>10</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>11.46%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>15(15.63%)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>11.46%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1.04%</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>96(100)</td>
</tr>
</tbody>
</table>

### Table 2

Antimicrobial susceptibility profiles of organisms isolate

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Escherichia coli</th>
<th>Citrobacter spp</th>
<th>Klebsiella pneumonia</th>
<th>Pseudomonas aeruginosa</th>
<th>Proteus mirabilis</th>
<th>Enterobacter spp</th>
<th>Staphylococcus aureus</th>
<th>Acinetobacter spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Bacterial pathogen</th>
<th>Total isolate</th>
<th>Number of MDR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>10</td>
<td>None</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>7</td>
<td>None</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>11</td>
<td>None</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>16 (16.7)</td>
</tr>
</tbody>
</table>

### DISCUSSION

Urinary tract infection (UTI) is commonly encountered and treated globally and the trend of uropathogens and their antibiotic susceptibility continue to change [13]. The overall prevalence of UTI in our work was 36.9% and this is consistent with 32% and 30.4% reported by Mane et al., [19] and Preethishree et al., [20] respectively all in India with a slight difference. Our value was lower than 67.2% reported by Abubakar [21], in Yola, 62.0% Anejo-Okopi et al., [13] in Maiduguri all in Nigeria. Our 36.9% prevalence was also higher than the 20.7% reported in Dhaka,Bangladesh [22], and 29. 3% in South West Nigeria reported by Ochada et al., [23]

Gender distribution showed male-female ratio of 1:2, Preethishree et al., [20] and Orhue [24] both reported 1:1.6 respectively. Rangari et al., [2] and Agbagwa and Ibeancho [25] also reported female preponderance in their studies. Higher prevalence in female could be attributed to the physiological and anatomical differences [19, 25].

Single pathogens were seen in all the significant culture growths and this was in consonance with most work done locally and internationally. The etiological agents identified in our work were similar to that of other researchers but with different isolation frequencies. The variations in the types and distribution of the uropathogens may be attributed to
different climatic condition including host factors, practices such as quality of healthcare and education levels, socioeconomic standard and hygiene practices in each country [2].

The gram negative bacteria constituted the largest group accounting for 84.4% of all UTI cases in our work while Staphylococcus aureus (15.6%) was the only Gram positive organism isolated. Among the Gram negative organism, E.coli (32.3%) ranked highest followed by Citrobacter spp (23.96%). This was consistent with the studies in Nigeria and other countries [2, 13, 19, and 20] but at variance with the work of Agbagwa and Ifeanacho [25] that reported Klebsiella spp as the commonest uropathogens. Knowledge of the antibiotic susceptibility profile among the uropathogen responsible for UTI is not only important in guiding clinicians to appropriate prescription of antibiotics, it also provides evidence based recommendations in empirical antibiotic therapy of UTI [26]. The result of this study demonstrated high sensitivity of gram negative bacteria to nitrofurantoin, tigecycline, impinem and meronem. Most of the isolates exhibited high resistance to augmentin, ceftazidime, oflaxacin doxycyclin, carbanicillin, and gentamycin prevalence of MDR was 27.39% in E.coli while 28.6% of Staph aureus isolated were methicillin resistant. Meticillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the mecA gene that allows the organism to grow and divide in the presence of meticillin and other β-lactam antimicrobials [27]. The mecA gene resides on the staphylococcal cassette. The ease of transfer of this genetic element demystify the increasing resistance to β-lactam antimicrobials such as penicillin and its chemical derivatives including cephalosporins [18, 28].

Resistance of the isolates to most of antimicrobials may have been due to earlier exposure of the organisms to these antimicrobial agents. Very many factors have been known to contribute to antibiotic resistance and these include inappropriate and baseless drug misuse by general practitioners in clinical practices, amateur practitioners, poor quality drugs, fake and expired drugs and the use of antibiotics without laboratory diagnosis are all serious contributing factors [29].

CONCLUSION

Gram negative bacteria are the major cause of UTI and antimicrobial resistance has become a significant public health problem in Nigeria. Periodic surveillance of antimicrobial susceptibility is imperative.
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